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Experimental Studies on Methionine as a Lipotropic Factor

VII. Summary, Discussion and Conclusions of the Whole Treatise

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Introduction

In spite of extensive studies hitherto-conducted, both physiological and clinical, little light has been thrown upon our knowledge of functions of the liver, including the rôle as played by the liver in the metabolism of enzymes. Above all, we have as yet very little information on the effect of one lipotropic factor,— methionine —, upon the variations of serum cholinesterase activity, in case of experimental hepatic impairment. It is a universally known fact that serum cholinesterase diminishes in case of the liver damage. And experimental evidence confirms the decrease of the activity after administration of hepatotoxic agents. However, the majority of present theories on the mechanism of the decrease are mere conjectures. And since the physiological significance of cholinesterase itself remains to be fully clarified, it may be difficult to expect a complete elucidation of this problem at this moment. However, clinical observations have often informed us that a lipotropic factor can prevent the liver from injury due to the administration of hepatotoxic agents, and that decreased serum cholinesterase activity, in cases of hepatic impairment, can often be reactivated at an earlier period. Therefore, it is conjectured that an experimental study on the effect of lipotropic factors upon decreased cholinesterase activity, due to the liver injury, may be an aid to the information of the physiological significance of serum cholinesterase and the mechanism of the beneficial action of lipotropic factors against liver injury. With this in view, the author has studied the effect of methionine on the experimental liver damage, 'pin-pointing' the course of fluctuation of the serum cholinesterase activity. Results of several liver function tests, fluctuation of serum protein, including the polarographic serum protein waves (PPW), and the changes in histological findings of the liver, have been studied simultaneously. Results were reported successively in the Sapporo Medical Journal, and in general meetings of the Japanese Gastroenterological Society. At present, withthe experiments completed, it is the author's intent to make a summarized discussion on the hitherto-obtained experimental results.

Summarized Results of the Whole Treatise

Prior to experimental studies, bibliographical discussions were made, concerning the problems related to the present study. In the present paper, redundancy shall be avoided, and the outline of tne results obtained by subsequent experiments are summarized as follows:

1) *Liver Function of Normal Dogs*: Prior to the commencement of the experiments, several liver function tests were performed on normal dogs.

i) Results of bromsulfalein test in normal

dogs are below 1%(average 0.16%), when the dye is injected (5mg per kg body weight) and the blood is withdrawn after 30 minutes.

ii) Since results of serum cobalt reaction were shifted much to the left as compared with human materials, the concentration of the cobalt reagent was duplicated for convenience's sake. Results deviate from R′5 to R′7.

iii) Serum cholinesterase activity in normal dogs, as determined by Hesterin-Miyazaki's method, is from 5.4 to 24.4 (average 10.70). This generally coincides with the data obtained by Ammon-Nachmansohn's Warburg technique.

iv) Serum alkaline phosphatase activity is from 1.2 to 5.6 (average 2.33) units.

v) A correlation to a certain degree was noted between the serous values of cholinesterase activity and of alkaline phosphatase activity. However, these enzymatic activities showed hardly any correlation to the results of serum cobalt reaction.

2) *Functional Change of the Injured Liver*: Functional changes of the liver when injury was inflicted by a single dose of chloroform (1 cc per kg body weight), were studied subsequently.

i) Bromsulfalein retention increases as a result of chloroform injection, until on the following day its 30 minute-value indicates retention from 6 to 25% (average 17.57%), followed by a gradual decrease with the lapse of time.

ii) Scarcely any set rule is noted in the changes of the results of serum cobalt reaction following the liver damage.

iii) Serum cholinesterase activity decreases as a result of the liver damage, while some of the cases show a temporary increase of the activity. The decreased cholinesterase activity then gradually returns to normal, with the lapse of time.

iv) Serum alkaline phosphatase activity is increased by the liver damage. The temporary decrease of the activity, as observed with cholinesterase, is rarely detectable.

3) *Effect of Methionine upon the Functional Changes of the Impaired Liver*: Functional change of the liver, due to the injury, in dogs with 30 g pre-feedings of DL-methionine, were studied subsequently.

i) With regard to bromsulfalein retention test and serum cobalt reaction, nothing could be ascribed as the special effect of methionine, as compared with the control.

ii) On the other hand, serum cholinesterase activity, in methionine-treated dogs, was revealed to have no phase of decline, which was observed in non-treated dogs. In some of the methionine-treated dogs even a tendency of the activity to rise was observed.

iii) Serum alkaline phosphatase activity is elevated also in methionine-treated dogs, but the degree of the increase is generally less significant.

4) *Effect of Methionine on the Changes of Serum Protein Components*: Results of investigation in the effects of methionine on the fluctuations of serum protein levels (refractometry) and changes in its components (electrophoresis), in cases of liver damage, are as follows:

i) Total protein level decreases on infliction of liver damage in which albumin plays the principal rôle.

ii) When methionine is administered to normal dogs, the total protein level is observed to increase. This increase mainly depends on α- and β-globulin. When hepatic damage is inflicted on said dogs, total protein level decreases. This is almost similar to the control, but the degree of the decrease is less significant.

5) *Effect of Methionine on the Changes of Polarographic Protein Waves*: Polarographic protein waves (PPW) were studied simultaneously. Decreased PPW-heights were observed after hepatic damage, which are not accompanied by any particular indication of the effect of methionine. Similarly, no specific correlations between PPW and

cholinesterase activity were noted.

6) *Effect of Methionine on the Histological Changes of the Liver*:

Changes in the histological findings of the liver were studied as finishing touches, which may serve as a morphological support of the above-mentioned functional changes.

i) As previously reported by many authors, the histological aspect of the liver on the day following the chloroform injection is manifested by a widespread fatty degeneration and necrosis which breaks out from the central portion of each lobule. This condition continues to remain after a week, with an appearance of regenerating liver cells in the central zones.

ii) There exists no marked histological change of the liver, which may be ascribed to the effect of methionine. After infliction of liver damage, histological changes of the liver in methionine-treated dogs are observed to resemble that of the control, but among the changes the degree of fatty degeneration is less remarkable as compared with the control animals.

iii) As to the vicissitude of liver cell RNA, no marked difference was observed between methionine-treated and nontreated animals.

Discussion

The information reported in the whole treatise is summarized and discussed as follows:

Following a single dose of subcutaneous chloroform administration (1cc per kg body weight), a widespread fatty degeneration and necrosis occurs. Functional disorders are represented by decreased cholinesterase activity, and increased alkaline phosphatase activity. Though bromsulfalein retention is aggravated, results of cobalt reaction are observed to be without pattern. Serum protein level is lowered by the liver damage, where albumin plays the principal rôle in the decrease. PPW is also lowered by the liver damage, and especially the lowering of the second wave is noteworthy.

Methionine-feeding to normal dogs does not alter the histological aspect of the liver. Functionally, bromsulfalein retention, cobalt reaction and alkaline phosphatase activity are also not altered. On the other hand, a marked increase of total protein level is observed, which is mainly due to the increase of α- and β-globulin.

When liver damage is inflicted on methionine-treated dogs, histological changes of the liver resemble that of the control, while the degree of fatty degeneration is less significant than in the control. Effect of methionine upon the functional changes of the liver is noted, especially in the course of serum cholinesterase activity, which is never accompanied by a decrease as observed in the control animals. Though the degree of alkaline phosphatase elevation and of serum protein depletion were observed to disagree more or less with the control, bromsulfalein retention (30 minute value), serum cobalt reaction and the changes in PPW-heights were almost the same in methionine-treated and non-treated animals.

It is surmised that the decrease of serum cholinesterase activity, in the case of liver damage, is due to the inhibition of the biosynthesis of the enzyme, which is carried out by the liver cells. Cajori & Vars[1] and Brauer & Root[2] observed that serum cholinesterase was synthesized by the liver cells, and serum cholinesterase activity therefore is considered to be a representative of other protein-biosyntheses within the liver. From this point of view, an opinion that serum cholinesterase has an intimate correlation with serum albumin

1) Cajori & Vars (1938): cit. in Lichtman, S. S.: Diseases of the Liver, Gall Bladder and Bile Ducts, 3 Ed. (Phila. 1953).

2) Brauer, R. W. & Root, M. A.: Am. J. Physiol. **149**, 611 (1947).

level, which is maintained by Alcalde[3], Levine & Hoyt[4] and Kunkel & Ward[5], may be accepted as seemingly appropriate. In fact, serum cholinesterase and albumin mostly have an intimate reciprocal relation. However, as discussed in the fifth issue of the present series, clinical and experimental observations have at times failed to demonstrate such mutual correlations, and there are not a few investigators, including Wilson[6], who have gainsaid such a correlation. According to recent information, cholinesterase itself is regarded as a component of α-globulin, rather of albumin. Various factors may influence the α-globulin level, and the level does not always follow a set fluctuation with varieties of hepatic damages. Alpha-globulin is said to be produced by liver cells[7], and a decrease of the α-globulin level in cases of hepatic insufficiency is reported. On the other hand, it is also noted that ensuing upon the destruction or inflammation of the tissue, α-globulin level may be elevated, owing principally to the increase of the mucoprotein fraction[8]. The distribution of α-globulin, in the case of liver diseases,may indicate an intricated aspect, owing to the participation of the above-designated factors. In the author's experiments, which have been reported in the fifth issue, serum cholinesterase was not observed to have any satisfactory correlation with serum protein fractions. This may rather be expected, in view of the discrepancy of sensitivity, between electrophoresis and the demonstration of enzymatic activity.

Various opinions have been expressed on the relation between the states of functional disorders and the localization of damage within the lobules. It may rather be expected that a variety of functional dsiorders are established according to the variety of the mode of damage. Alpha-globulin itself may perhaps be a combination of many components with different habitats and different modes of production, and therefore each of the components may have a difierent trend when a hepatic injury is inflicted. Among the α-globulin components, cholinesterase as far as the present study is concerned, is especially sensitive to methionine, and the decline of tho serous level of the enzyme was observed to be checked almostcompletely by previously administered methionine. As to the electrophoretical analysis of the serum, α-globulin did not seem to supply sufficient evidence of a parallelism with cholinesterase activity, owing to the complexity of the other components of this fraction, or owing to the intricated fluctuation of the other protein fractions. In connection with this problem, the author investigated the changes of PPW-heights, which is considered to have an intimate connection with serum protein fractions. Declines of PPW-heights were observed both in methionine-treated and non-treated dogs, and nothing was found which could be attributed to the virtues of methionine. According to Satō in the author's own research institute, there is a certain parallelism between PPW and serum α-globulin level. In the author's present study, the fluctuation of serum cholinesterase activity did not seem to have any direct parallelism with PPW or with α-globulin lebel, as far as the present experimental conditions are concerned.

The following may serve as a conclusion of the above-discussed problems:

Cholinesterase is possibly synthesized by the liver, under a more or less independent mechanism of ot her serum protein syntheses, and the cholinesterase synthesis may be checked by liver damages, but is subject to the pro-

3) Alcalde, O.: J. Lab. & Clin. Med. **33**, 11 (1948).
4) Levine, M. G. & Hoyt, R. E.: Science **111**, 286 (1950).
5) Kunkel, H. O. & Ward, S. M.: J. Exper. Med. **86**, 325 (1947).
6) Wilson, A. et al.: J. Clin. Invest. **31**, 815 (1952).
7) Roberts, S. & White, A.: J. Biol. Chem. **180** (1949).
8) Greenspan, E. M. et al.: J. Lab. & Clin. Med. **39**, 44 (1952).

tective efficacy whichis conducted by methionine. Methionine, in this case, may also act as a SH-donor, as Witts[9] has asserted, in addition to its original action as a lipotropic factor.

As it is the author's intent, aside from the present study, to make a histochemical demonstration of cholinesterase, additional studies will be made to determine above-mentioned problems, together with the use of electrophoretical analysis of α-globulin subfractions, SH-group estimation, etc.

Conclusions

Liver function in normal dogs and changes in the liver function, in case of infliction of liver damage were studied, together with the effects of methionine on said changes after methionine prescription. Results are summarized as follows:

1) Functional states of the liver in normal dogs are reported in the second issue of the present series. Serum protein construction in normal dogs are reported in the fifth issue.

2) As a result of the liver damage, bromsulfalein retention is augmented, and serum alkaline phosphatase level is elevated. Serum cholinesterase activity, on the contrary, is diminished by the liver damage. Serum cobalt reaction does not follow a certain rule. The most important rôle in serum protein depletion is played by the decrease of albumin content.

3) In methionine-treated dogs, bromsulfalein retention does not difier greatly from the control. Similarly, changes in the results of serum coblat reaction does not indicate any set tendency which is identical to the control. Increase of serum alkaline phosphatase activity is observed to be somewhat less significant than the control, while serum cholinesterase activity indicated no sign of reduction. The last finding is a significant discrepancy between methionine-treated and non-treated groups. Serum protein level increases after methionine-prescriptions. Augmentation of α-and β-globulin levels is the principal damage. Total protein level falls again by the liver damage, but the degree of the reduction is less significant than the control.

4) Histological changes of the liver roughly coincides with hitherto-reported cases, except in one point where fatty degeneration is less remarkable in methionine-treated dogs, as compared with the control. No sign of discrepancy, between methionine-treated and non-treated groups, was observed in regard to the liver cell RNA.

5) Nothing can be attributed to the virtue of methionine on the decline of PPW-heights due to liver damage.

6) The above-enumerated results lead to the following conclusions:

Biosynthesis of cholinesterase, due to liver cells, does not run quantitatively parallel to the biosyntheses of other proteins. Inhibition of cholinesterase-biosynthesis, due to the liver damage, is readily prevented by methionine-prescription.

Though the mechanism of this prevention remains obscure, an efficacy of methionine in the capacity of SH-donor must not be neglected together with its original lipotropic effect.

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9) Witts, L. L.: Brit. Med. J. 1, 145 (1947).